Original Article

Family History of Diabetes Links Impaired Substrate Switching and Reduced Mitochondrial Content in Skeletal Muscle

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Insulin resistance is associated with metabolic inflexibility, impaired switching of substrate oxidation from fatty acids to glucose in response to insulin. Impaired switching to fat oxidation in response to a high-fat diet (HFD) is hypothesized to contribute to insulin resistance. The objective of this study was to test the hypothesis that defects in substrate switching in response to insulin and a HFD are linked to reduced mitochondrial biogenesis and occur before the development of diabetes. Metabolic flexibility was measured in young sedentary men with (n = 16) or without (n = 34) a family history of diabetes by euglycemic-hyperinsulinemic clamp. Flexibility correlated with fat oxidation measured in a respiratory chamber after a 3-day HFD. Muscle mitochondrial content was higher in flexible subjects with high fat oxidation after a HFD and contributed 49% of the variance. Subjects with a family history of diabetes were inflexible and had reduced HFD-induced fat oxidation and muscle mitochondrial content but did not differ in the amount of body or visceral fat. Metabolic inflexibility, lower adaptation to a HFD, and reduced muscle mitochondrial mass cluster together in subjects with a family history of diabetes, supporting the role of an intrinsic metabolic defect of skeletal muscle in the pathogenesis of insulin resistance. *Diabetes* 56:720–727, 2007

A high-fat diet (HFD) is a risk factor for obesity and has been implicated in the development of insulin resistance (1). A short-term HFD, as well as a lipid infusion, causes insulin resistance (2–4), reduces oxidative metabolism in skeletal muscle in rats (5), and downregulates genes of oxidative phosphorylation and mitochondrial biogenesis, such as peroxisome proliferator–activated γ coactivator-1α (*PGC-1α*) (6). Moreover, oxidative phosphorylation and *PGC-1α* gene expression are decreased in insulin resistance (7,8). Taken together, these findings point to HFD, reduced oxidative capacity, and lipotoxicity in the pathophysiology of insulin resistance (9,10).

Substantial interindividual variability exists in the change of fat oxidation during adaptation to a HFD (11). Impaired fat oxidation during adaptation to a HFD is observed in restrained eaters (12), postobese (13) and obese (14) individuals, and in individuals with a family history of obesity (15), pointing toward a possible genetic basis for reduced fat oxidation (1). Impaired substrate switching in response to insulin (metabolic inflexibility) and dietary stimuli (attenuated adaptation to a HFD) are hypothesized to contribute to obesity and insulin resistance (1,16).

Metabolic inflexibility, as defined by Kelley and Mandarino (17), represents impaired substrate switching in skeletal muscle in insulin resistance. Healthy lean individuals who are flexible rely on lipids as a main source of fuel under fasting conditions and readily switch to carbohydrate oxidation in response to insulin infusion (18). On the contrary, the inflexible muscle of an insulin-resistant individual is characterized by lower fasting lipid utilization and lacks the ability to switch to carbohydrate oxidation in the insulin-stimulated state (18). Substrate competition in skeletal muscle and its role in systemic fatty acid utilization has been explored by a number of investigators (18,19).

Mitochondrial oxidative enzyme activity is reduced in skeletal muscle in obesity and insulin resistance (20). Bruce et al. (21) found that skeletal muscle oxidative capacity was a better predictor of insulin sensitivity than intramyocellular lipids. A higher fasting respiratory quotient (RQ), indicating decreased fat oxidation, is a predictor of weight gain (22). We showed that dynamic changes in fat oxidation in primary human muscle cells exposed to increased concentrations of fatty acid or glucose in vitro were closely related to metabolic flexibility, insulin sensitivity, and other clinical phenotypes of young, healthy donors in vivo (23), suggesting the importance of intrinsic, genetically, or epigenetically determined characteristics of fat oxidation for the flexible, insulin-sensitive phenotype. Decreased mitochondrial ATP synthesis in the offspring of patients with type 2 diabetes (24) supports the hypothesis that genetic factors control the reduced mitochondrial oxidative phosphorylation of insulin-resistant individuals.

Type 2 diabetes is largely a genetic disorder with a strong environmental influence (25). The concordance for type 2 diabetes in siblings is very high (26), and offspring

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FFA, free fatty acid; mtDNA, mitochondrial DNA; HFD, high-fat diet; RQ, respiratory quotient.

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of a parent with type 2 diabetes have reduced insulin sensitivity (27) and increased risk of type 2 diabetes (28). The difference in weight gain during a HFD (15) and in overfeeding twin studies (29) supports the role of genetic make-up in the adaptation to a HFD.

Taken together, these data suggest that the ability to oxidize dietary fat plays a role in the pre-diabetic condition and has a genetic predisposition. The aim of this study was to test the hypothesis that impaired substrate switching from carbohydrate to fat oxidation in response to a HFD is, together with metabolic inflexibility and reduced mitochondrial content, manifestation of an underlying disorder intrinsic to skeletal muscle, which contributes to the pathogenesis of diabetes. We measured these three parameters in 50 healthy sedentary young men with a range of body compositions and with family history of type 2 diabetes. In subjects with a family history of type 2 diabetes, before overt hyperglycemia, these three defects cluster together, forming a triad that may increase the risk of diabetes.

RESEARCH DESIGN AND METHODS

The ADAPT Study is a short-term, cross-sectional interventional study designed to examine interindividual differences in fat oxidation on an isoenergetic HFD. The protocol was approved by the Institutional Review Board of the Pennington Biomedical Research Center. All volunteers gave written informed consent. Healthy young sedentary men, aged 18–29 years, with a BMI 20–35 kg/m^2 underwent complete physical examination, routine medical laboratory tests, and anthropometry. After completing the screening process, participants presented at the Pennington In-Patient Unit on day −4 and ate a weight-maintaining diet consisting of 35% fat, 16% protein, and 49% carbohydrate. On day −3, a euglycemic-hyperinsulinemic clamp was performed. On day 1, participants entered a respiratory chamber and ate the same weight-maintaining 35% fat diet. On days 2–4 (3 days) in the respiratory chamber, participants were fed an iso-energetic HFD (50% fat, 16% protein, and 34% carbohydrate). Total daily energy expenditure, fat oxidation, protein oxidation, and carbohydrate oxidation were measured at energy balance using an algorithm to balance intake and expenditure within the day (30). A family history of diabetes was determined by questionnaire.

Euglycemic-hyperinsulinemic clamp. Insulin sensitivity was measured by euglycemic-hyperinsulinemic clamp (31) before a HFD. Subjects were asked to refrain from vigorous physical activity for 48 h before the clamp. After an overnight fast, insulin (80 mU/m^2 per min) and 20% glucose (to maintain plasma glucose at 90 mg/dl) were administered for 3 h. Glucose and insulin were measured in triplicate blood samples 5 min apart at baseline and during steady-state insulin from 165 to 180 min. The glucose disposal rate, a measure of insulin sensitivity, was adjusted for kilograms of lean body mass determined by dual-energy X-ray absorptiometry. Fasting RQ, postinsulin steady-state RQ (clamp RQ), and substrate oxidation of fat and carbohydrate were calculated from O_2 consumption and CO_2 production, measured before the clamp (fasting values) and during the steady state of the clamp by indirect calorimetry and corrected for nitrogen excretion. A total of 48 individuals completed the clamp. Due to problems during the clamp procedure, such as failed intravenous needle insertions, three outliers with fasting RQ >3 SD were excluded from the analysis of the clamp RQ. The insulin-stimulated change in RQ (ΔRQ clamp = clamp RQ − fasting RQ), a measure of metabolic flexibility, represents the ability to switch from fasting fat oxidation to insulin-stimulated carbohydrate oxidation and is decreased in insulin-resistant states (17). Subjects were divided into three categories based on the work of Kelley et al. (18): flexible ΔRQ >0.13 (n = 10), inflexible ΔRQ <0.06 (n = 12), and intermediate ΔRQ 0.06–0.13 (n = 23).

Maximal aerobic capacity. Maximal oxygen uptake ($V_{O_{2max}}$) was determined by a progressive treadmill test to exhaustion in accordance with the recommendations described by the American College of Sports Medicine (32). The volume of O_2 and CO_2 were measured continuously using a metabolic cart (V-Max29 Series; SensorMedics, Yorba Linda, CA). Heart rate was continuously monitored using a portable heart rate monitor (Polar S-610; Polar Beat).

Body composition. Body fat mass and lean body mass were measured on a Hologic Dual Energy X-ray Absorptiometer in the fan beam mode (QDR 4500; Hologic, Waltham, MA). Coefficient of variation for the measurement of the lean body mass, fat mass, and percentage of body fat is 0.8, 1.6, and 1.7%, respectively. Visceral fat was measured by computed tomography scanning using a GE High Speed CT scanner under an established protocol (33).

Respiratory chamber. The 24-h energy expenditure and RQ were determined in a whole-room respiratory calorimeter as previously described (30). O_2 and CO_2 concentrations were measured using a Magnos 4G magneto-pneumatic oxygen analyzer and Uras 3G infrared CO_2 analyzer (Hartmann and Braun). Energy expenditure and substrate oxidations were calculated from O_2 consumption, CO_2 production, and 24-h urinary nitrogen excretion using the equations of Acheson et al. (34). Sleep RQ, a measure of maximal fat oxidation, was measured from 2:00 to 5:00 A.M. Fat balance was calculated as a difference between fat intake and fat oxidation measured over the 3 days of a HFD. The reproducibility of RQ measurements was tested in 17 subjects who had a 24-h RQ measured on two different admissions. Interindividual variability of 24-h RQ and sleep RQ in 49 individuals was compared with intraindividual variability in 17 individuals (online appendix Figure [available at http://dx.doi.org/10.2337/db06-0521]), as described by Zurlo et al. (22). Intraindividual variability was in all cases lower than interindividual variability.

Triaxial accelerometry. Free-living energy expenditure was assessed by accelerometer (Actigraph, Fort Walton Beach, FL). The participant wore the device on the wrist for 3 consecutive days, including 2 weekdays and 1 weekend day. The device was validated against doubly labeled water showing an R^2 of 0.65 ($P < 0.001$) between the two methods (J. Delany, L.d.J., unpublished observation). Measurements were completed for 24 subjects (n = 15 for family history negative and n = 9 for family history positive subjects). Energy spent by habitual physical activity was calculated as 24-h energy expenditure by accelerometer/resting energy expenditure by indirect calorimetry.

Skeletal muscle biopsy was performed before (day −2, on a standard diet) and after 3 days on a HFD (day +5). Samples of vastus lateralis muscle, weighing 60–100 mg, were obtained by muscle biopsy using the Bergstrom technique (35) and snap frozen in liquid nitrogen.

DNA extraction. DNA from 50–100 mg of vastus lateralis muscle was extracted with phenol-chloroform, after separation of protein and RNA with Trizol reagent. The total amount of DNA recovered was determined by spectrophotometry.

Real-time PCR for mitochondrial DNA. Relative amounts of nuclear DNA and mitochondrial DNA (mtDNA) were determined by quantitative real-time PCR as previously described (36). The sequences for the primer/probe sets used in TaqMan analysis of mtDNA content for NADH dehydrogenase subunit 1 are forward primer CCCTAAAACCCGCCACATCT, probe CCATCAC CCTCTACATCACCGCCC, and reverse primer GAGCGATGGTGAGAGCTA AGGT and for lipoprotein lipase (accession no. NM_000237) are forward primer CGAGTCGTCTTTCTCCTGATGAT, probe ACATTCACCAGAGGGTC, and reverse primer TTCTGGATTCCAATGCTTCGA.

Statistical analysis. All clinical data were entered into the Pennington Biomedical Research Center database, extracted and combined with a database that included mtDNA data, and analyzed using SAS (version 8.2) and JMP version 5.0.1a (SAS, Cary, NC). Since all variables were normally distributed, correlations were performed in a pairwise fashion using the Pearson product-moment statistic. A two-sample t test was performed to compare subjects with a negative and positive family history of diabetes. Regression analysis with stepwise model selection was used to determine the best determinant of sleep fat oxidation on the 3rd day of a HFD. All values are presented in figures and tables as sample (raw) means ± SD or SE. The type II error rate was set a priori at $P < 0.05$.

RESULTS

The characteristics of the study population are presented in Table 1. In general, subjects were young, healthy, sedentary men with a broad range of BMI (20.1–34.7 kg/m^2), fatness (10.5–32.3%), and insulin sensitivity (glucose disposal rate 4.03–24.5 mg/kg lean body mass/min). Sixteen subjects had a parent (n = 9) or a grandparent (n = 7) with diabetes, while 34 subjects had no family history of diabetes.

Relationship between metabolic flexibility and insulin sensitivity. As expected, glucose disposal during the clamp, a measure of insulin sensitivity, correlated negatively with BMI, fat mass, and percent body fat and visceral fat but correlated positively with $V_{O_{2max}}$ and mtDNA (Table 2), confirming the well-documented relationship between body fatness, fitness, and insulin sensitivity (37). A negative correlation was observed between insulin sensitivity and fasting levels of both insulin and free fatty acids (FFAs) on a 35% fat diet (Table 2). Fasting

TABLE 1
Clinical characteristics of the study population

Phenotype	Overall	Family history of diabetes		*P* value	Range
		Positive	Negative		
Ethnicity (Caucasian/African American/Asian)	38/6/6	13/2/1	25/4/5		
Age (years)	22.3 ± 2.9	22.3 ± 2.1	22.3 ± 3.3	NS	18–29
Body weight (kg)	81.3 ± 12.4	80.2 ± 13.8	81.8 ± 11.9	NS	59–118
BMI (kg/m^2)	26.1 ± 3.9	25.9 ± 4.5	26.2 ± 3.7	NS	20.1–34.7
Body fat (kg)	16.8 ± 7.0	17.0 ± 8.7	16.8 ± 6.2	NS	7.3–34.6
Body fat (%)	20.3 ± 6.4	20.3 ± 7.5	20.2 ± 5.8	NS	10.5–32.3
Visceral fat (kg)	2.1 ± 1.3	2.1 ± 1.3	1.9 ± 1.2	NS	0.6–5.4
Vo_{2max} (ml/kg LBM/min)	41.5 ± 7.5	39.2 ± 7.5	42.5 ± 7.4	NS	23.5–59.2
Insulin sensitivity (mg/kg LBM/min)	11.0 ± 4.2	10.1 ± 1.2	11.5 ± 0.7	NS	4.0–24.5
Fasting RQ (clamp)	0.84 ± 0.38	0.83 ± 0.02	0.84 ± 0.04	NS	0.74–0.95
ΔRQ (clamp RQ − fasting RQ)	0.090 ± 0.045	0.077 ± 0.034	0.098 ± 0.049	NS	0.030–0.250
24-h RQ STD (30% fat)	0.87 ± 0.02	0.87 ± 0.02	0.87 ± 0.02	NS	0.83–0.93
24-h RQ HFD (50% fat)	0.83 ± 0.01	0.83 ± 0.03	0.83 ± 0.03	NS	0.81–0.87
Sleep RQ STD	0.84 ± 0.03	0.85 ± 0.07	0.84 ± 0.005	NS	0.72–0.91
Sleep RQ HFD	0.81 ± 0.02	**0.824 ± 0.018**	**0.805 ± 0.022**	**0.016***	0.77–0.84
Fat balance (g/3 days of a HFD)	−2.3 ± 40.6	−0.96 ± 30.2	−2.9 ± 45.0	NS	−105.2 to 96.4
Fasting glucose STD (mmol/l)	5.1 ± 0.4	5.1 ± 0.4	5.0 ± 0.4	NS	4.3–5.9
Fasting insulin STD (IU/ml)	10.6 ± 6.3	11.4 ± 7.7	10.3 ± 5.6	NS	3.1–26.8
Triglyceride STD (mg/dl)	125.8 ± 73.1	128.0 ± 70.7	127.1 ± 57.3	NS	36.0–328.0
Adiponectin STD (μg/ml)	5.9 ± 3.5	5.1 ± 3.7	6.1 ± 3.4	NS	1.6–16.8
Leptin STD (ng/ml)	6.2 ± 5.3	7.0 ± 7.2	5.8 ± 4.1	NS	1.0–27.0
FFA STD (mmol/l)	0.33 ± 0.11	0.36 ± 0.10	0.31 ± 0.11	NS	0.08–0.56
mtDNA copy number	1,260 ± 397	**1,065 ± 343**	**1,357 ± 392**	**0.018†**	590–2,276
Habitual physical activity	1.54 ± 0.1	1.51 ± 0.13	1.57 ± 0.09	NS	1.4–1.8

Data are means ± SD. Unpaired *t* test was performed to test for the effect of family history of diabetes. $n = 16$ with a family history of diabetes; $n = 34$ without a family history of diabetes. Insulin sensitivity was the glucose disposal rate (clamp) ($n = 32$ without a family history of diabetes). ΔRQ was the insulin-stimulated change in RQ (clamp). The 24-h RQ was measured in the respiratory chamber during the 3rd day of a standard (STD) or a HFD. Sleep RQ was measured in a respiratory chamber from 2:00 to 5:00 a.m., on the 3rd day of a standard or a HFD ($n = 33$ without a family history of diabetes). Habitual physical activity ($n = 9$ with a family history of diabetes; $n = 15$ without a family history of diabetes). Total daily energy expenditure by accelerometer (kcal/day)/resting energy expenditure by indirect calorimetry (kcal/day). Significant results are presented in boldface. *$P = 0.012$ after adjusting for Vo_{2max}; †$P = 0.06$ after adjusting for Vo_{2max}. LBM, lean body mass.

RQ, a measure of fasting substrate utilization before the clamp, was only related to fasting FFA but not to insulin sensitivity or percent body fat. Metabolic flexibility, defined as the insulin-stimulated change in RQ during the clamp (ΔRQ; Fig. 1*A*), was negatively correlated with body fat, indicating a lower capacity for substrate switching in subjects with higher fat mass, specifically with higher visceral fat (38) (Table 2). On the other hand, ΔRQ was positively correlated with insulin sensitivity and Vo_{2max}, demonstrating the relationship between metabolic flexibility, insulin sensitivity, and aerobic fitness, respectively (Table 2). Sleep RQ on a 35% fat diet was inversely related

TABLE 2
Insulin sensitivity, metabolic flexibility, and maximal HFD-induced fat oxidation; relationship to clinical characteristics and mtDNA content in skeletal muscle

Clinical phenotype	Sleep fat oxidation (day 3 of an iso-energetic 50% fat diet)	Substrate utilization before and during a clamp on a standard diet (35% fat)		Insulin sensitivity on a standard diet (35% fat)
	Sleep RQ HFD	ΔRQ	Fasting RQ	Glucose disposal
BMI (kg/m^2)	0.105	−0.220	−0.106	−0.343*
Body weight (kg)	0.073	0.005	−0.186	−0.231
Fat mass (kg)	0.072	−0.320*	−0.135	−0.579†
Body fat (%)	0.043	−0.400*	−0.084	−0.641†
Visceral fat (kg)	0.087	−0.350*	−0.014	−0.495†
Insulin sensitivity (mg/kg LBM/min)	−0.208	0.640†	0.205	—
Vo_{2max} (ml · kg^{-1} · min^{-1})	**−0.320***	0.360*	0.151	0.541*
Fasting FFAs (mmol/l)	0.099	−0.31	**−0.616†**	−0.620†
Fasting insulin (IU/ml)	0.08	−0.62†	0.14	−0.63†
mtDNA content	**−0.560†**	0.720†	0.080	0.580†

Data are *R* values. $n = 50$ except sleep RQ HFD ($n = 49$), insulin sensitivity ($n = 48$), metabolic flexibility ($n = 45$), and mtDNA content ($n = 45$). *$P < 0.05$; †$P < 0.005$. Significant results are in boldface. LBM, lean body mass.

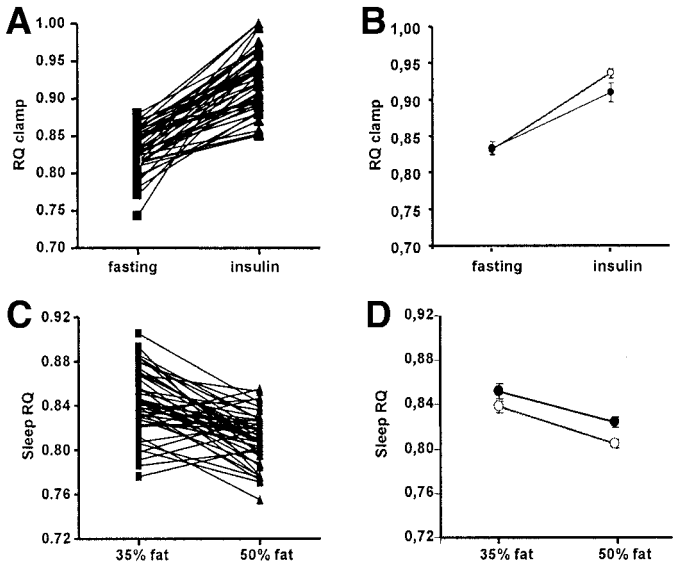

FIG. 1. ***A*****: Metabolic flexibility, an insulin-stimulated change in RQ during the clamp (n = 45). ΔRQ = clamp RQ − baseline RQ.** ***B*****: Metabolic flexibility in subjects with ● or without ○ a family history of diabetes.** ***C*****: Maximal HFD-induced fat oxidation (n = 49), the decrease in sleep RQ measured from 2:00 to 5:00 A.M. during the 3rd day of a HFD (chamber).** ***D*****: Higher sleep RQ on the 3rd day of a HFD points to lower maximal HFD-induced fat oxidation in subjects with ● versus without ○ a family history of diabetes.**

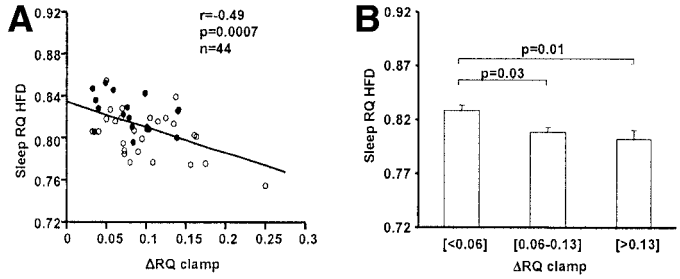

FIG. 2. ***A*****: Maximal HFD-induced fat oxidation (sleep RQ on the 3rd day of a HFD) is related to metabolic flexibility (ΔRQ clamp).** ***B*****: Maximal HFD-induced fat oxidation is lower in individuals with metabolic inflexibility (ΔRQ clamp <0.06) (n = 12) compared with more flexible individuals (0.06 < ΔRQ clamp > 0.13, n = 23; ΔRQ clamp >0.13, n = 10) (18). ●, subjects with a family history of diabetes; ○, subjects without a family history of diabetes.**

to insulin sensitivity (r = −0.32, P = 0.03), indicating a link between insulin action and impaired fat oxidation. The relationship was weakened after adjustment of insulin sensitivity to body fat (P = 0.1, data not shown).

Adaptation to an iso-energetic high-fat, low carbohydrate diet. As expected, we found a decrease in 24-h RQ ($P < 0.0001$) and a decrease in sleep RQ ($P < 0.0001$) on the 3rd day of the HFD (Fig. 1*B*), demonstrating substrate switching toward fat oxidation. Sleep RQ, measured from 2:00 to 5:00 A.M., is undisturbed by physical activity and food intake/thermic effect of food. Thus, the sleep RQ measures the minimal RQ and the maximal sleep fat oxidation.

Maximal fat oxidation after 3 days of a HFD (sleep RQ) is correlated with metabolic flexibility. To determine whether there is a relationship between fat oxidation on a HFD and metabolic flexibility (clamp), we examined substrate oxidation, measured by 24-h RQ and sleep RQ, both measured on the last day of the 50% fat diet and cumulative fat balance over 3 days of the HFD in relation to metabolic phenotypes. We found that sleep RQ during the HFD was inversely related to metabolic flexibility (ΔRQ clamp) (Fig. 2), identifying a link between an insulin-stimulated change in substrate switching and sleep fat oxidation. This relationship remained significant after eliminating an outlier with ΔRQ clamp 0.25 (>3 SD) (r = −0.31, P = 0.03). Sleep RQ during the HFD was positively correlated with the aerobic capacity ($V\text{O}_{2\text{max}}$) (r = −0.28, $P < 0.05$). Total (oxidative and nonoxidative) glucose disposal during the clamp did not correlate with sleep RQ during the HFD (P = 0.15), suggesting that insulin-stimulated changes in substrate oxidation (oxidative glucose disposal) rather than total insulin-stimulated glucose uptake links sleep RQ with metabolic flexibility. We did not find a significant relationship between 24-h RQ on a HFD and metabolic phenotypes (metabolic flexibility P = 0.32; insulin sensitivity P = 0.8; $V\text{O}_{2\text{max}}$ P = 0.8).

As expected, cumulative fat balance over 3 days of a HFD correlated positively with 24-h RQ and sleep RQ on the 3rd day of a HFD (Table 3), demonstrating increased fat storage in response to a HFD in less adaptable subjects who are unable to increase fat oxidation (and to decrease RQ) sufficiently to match the fat content of the diet. We did not find any significant relationships between the change in 24-h RQ or sleep RQ on a HFD, calculated as the difference between RQ on a 35% fat diet and RQ on the 3rd day of a 50% fat diet, and insulin sensitivity, metabolic flexibility, $V\text{O}_{2\text{max}}$, body fat, et cetera.

Metabolic phenotypes are linked to mtDNA content in skeletal muscle. As expected, mtDNA content in skeletal muscle, normalized to nuclear DNA, did not change with a short 3-day 50% fat diet (P = 0.54). A positive correlation between mtDNA content before and after the HFD (r = 0.84, $P < 0.0001$, n = 30) demonstrated the reproducibility of the assay. mtDNA content was inversely associated with BMI (r = −0.37, P = 0.01) and body fatness (r = −0.33, P = 0.025), illustrating the lower mitochondrial number in skeletal muscle from subjects with a higher fat mass. There was a positive correlation between mtDNA content and aerobic capacity ($V\text{O}_{2\text{max}}$) (r = 0.41, P = 0.005). mtDNA content also correlated positively with metabolic flexibility and insulin-stimulated glucose disposal (Fig. 3*A* and *B*; Table 2) and correlated inversely with sleep RQ during a HFD (Fig. 3*C*), pointing out the importance of mitochondria for insulin responsiveness, as well as the role of mitochondria in substrate switching from fat to carbohydrate oxidation in response to a HFD. Fasting RQ before the clamp was not related to mtDNA content (P = 0.58). Multiple stepwise regression analysis, with sleep RQ during a HFD as the dependent variable and mtDNA content, $V\text{O}_{2\text{max}}$, ΔRQ, percent body fat, fasting insulin, and FFAs as independent variables, identified mtDNA content as the primary determinant of

TABLE 3
The 24-h RQ and sleep RQ on day 3 of an iso-energetic HFD are positively correlated to cumulative fat balance over 3 days of a HFD

	24-h RQ HFD	Sleep RQ HFD
Sleep RQ HFD	**0.680***	—
Fat balance (g/3 days of HFD)	**0.684***	**0.356†**

Data are IR values. n = 49. *$P < 0.005$; †$P < 0.05$. Significant results are boldface.

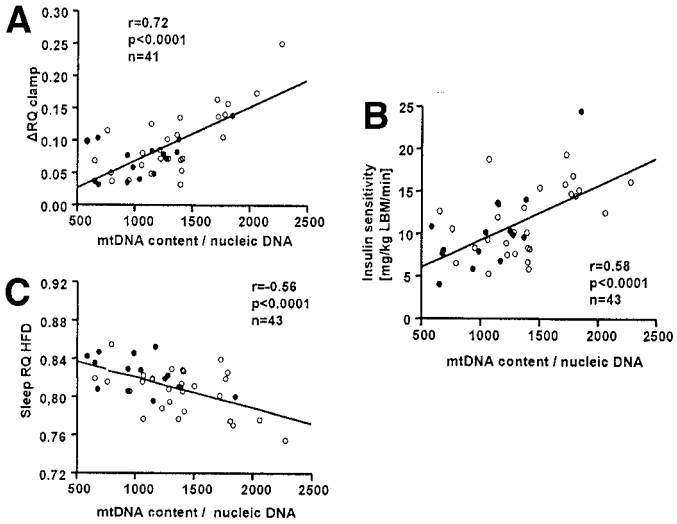

FIG. 3. mtDNA content in skeletal muscle is related to metabolic flexibility (ΔRQ clamp) (*A*), insulin sensitivity (clamp) (*B*), and maximal HFD-induced fat oxidation (*C*), a decrease in sleep RQ on the 3rd day of a HFD (chamber). DNA was isolated from vastus lateralis muscle. mtDNA content was normalized to nucleic DNA (qt PCR). ●, subjects with a family history of diabetes; ○, subjects without a family history of diabetes.

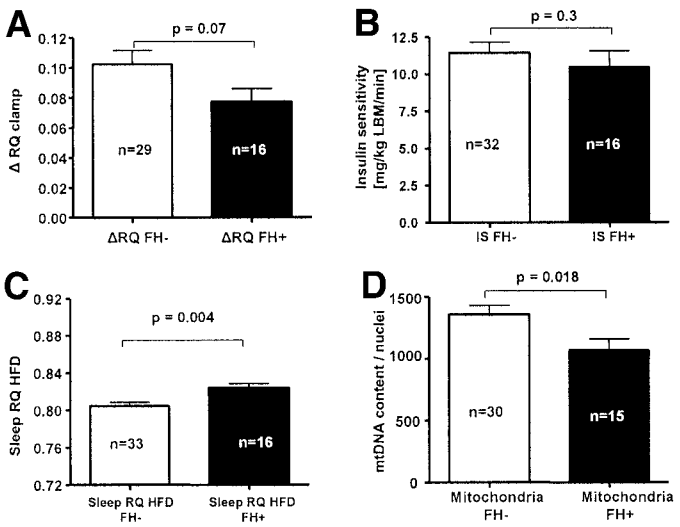

FIG. 4. Subjects with a family history of type 2 diabetes (FH) have reduced metabolic flexibility (ΔRQ clamp) (*A*); insulin sensitivity (IS) (*B*); maximal HFD-induced fat oxidation, demonstrated by a higher sleep RQ on the 3rd day of a HFD (*C*); and mtDNA content in skeletal muscle, normalized to nucleic DNA (*D*). Unpaired *t* test was performed to compare the groups with and without a family history of diabetes. ■, subjects with a family history of diabetes; □, subjects without a family history of diabetes.

sleep RQ during a HFD ($P = 0.003$), explaining 49% of the variance. mtDNA was inversely related to insulin and leptin before and after a HFD (insulin 35% fat diet: $r = -0.45$, $P = 0.002$; insulin 50% fat diet: $r = -0.46$, $P = 0.0016$; leptin 35% fat diet: $r = -0.43$, $P = 0.003$; leptin 50% fat diet: $r = -0.37$, $P = 0.01$), fasting FFAs (clamp) ($r = -0.31$, $P = 0.06$), FFAs during insulin infusion (clamp) ($r = -0.33$, $P = 0.06$), and FFAs on a 35% fat diet ($r = -0.31$, $P = 0.04$). mtDNA content was positively associated with plasma adiponectin after a HFD ($r = 0.36$, $P = 0.036$). No significant relationship was observed between mtDNA content, glucose, and fasting FFAs on a HFD.

Subjects with a family history of diabetes have impaired substrate switching, insulin resistance, and reduced mtDNA content in skeletal muscle. To test the contribution of genetic background to substrate switching and mtDNA content, we divided the study population into two groups: subjects with a family history of diabetes in a parent or grandparent ($n = 16$) and subjects without a family history of diabetes ($n = 34$). To check for the between-group differences, we used the unpaired *t* test (Table 1). Subjects with a family history of diabetes had a trend toward reduced metabolic flexibility ($P = 0.07$) (Fig. 4*A*) and had significantly higher sleep RQ on the 3rd day of a HFD ($P = 0.004$; $P = 0.012$ when adjusted for $V\text{O}_{2\text{max}}$), indicating lower maximal HFD-induced fat oxidation (Fig. 4*C*). We found a decrease in mtDNA content in subjects with a family history of diabetes ($P = 0.018$; $P = 0.06$ when adjusted for $V\text{O}_{2\text{max}}$) (Fig. 4*D*). We did not find a significant difference between the groups in BMI, body fat, or 24-h RQ before or after HFD (Table 1). Using triaxial accelerometry, we did not observe differences in habitual physical activity level ($P = 0.2$) in subjects with compared with those without a family history of type 2 diabetes.

DISCUSSION

Skeletal muscle displays a considerable plasticity in substrate switching between oxidation of carbohydrate and fat (39). In obesity, diabetes, and insulin resistance, patterns of substrate selection are altered and include metabolic inflexibility and defects of fat oxidation (16,17). Our studies indicate that defects in substrate switching, which cluster together with mitochondrial defects in young, sedentary, healthy men with a family history of type 2 diabetes, might be a manifestation of an underlying mitochondrial disorder(s) and intrinsic to skeletal muscle. This occurs before the onset of impaired glucose tolerance, suggesting that these defects are one of the primary factors implicated in the pathophysiology of type 2 diabetes.

Kelley and Mandarino (17) defined metabolic flexibility as the "acute" switch in substrate from fasting fat oxidation to insulin-stimulated carbohydrate oxidation during an insulin infusion. Our results demonstrate that defects in substrate switching, when dietary fat is increased, are linked to metabolic inflexibility. Furthermore, these two aspects of substrate switching, namely metabolic inflexibility and defects in substrate switching when dietary fat is increased, are linked with lower insulin sensitivity and lower mtDNA content in skeletal muscle; this forms a cluster of defects in subjects with a family history of type 2 diabetes.

An iso-energetic, high-fat, low-carbohydrate diet induces slow adaptive changes in substrate switching, as previously shown (11). We found that both sleep and 24-h fat oxidation after 3 days of a HFD were negatively correlated with fat balance over 3 days of a HFD. This leads to increased fat storage, a finding that is consistent with previous reports (11,22). A slower rate of adaptation to a HFD might translate into differences in weight gain over time, especially when combined with a sedentary lifestyle. Flatt (40) proposed that subjects who continue to oxidize carbohydrate in the postabsorptive state deplete carbohydrate stores; this leads to increased food intake to replace the diminished carbohydrate stores and lipid stor-

age. This is consistent with other prospective data (22). Through these mechanisms, interindividual differences in substrate selection might play a role in the development of obesity and subsequently type 2 diabetes.

In insulin resistance, skeletal muscle displays metabolic inflexibility, described as the impaired ability to switch from fat oxidation during fasting to insulin-stimulated carbohydrate oxidation (17). We show that metabolic flexibility parallels insulin-mediated glucose disposal and $V_{O_{2max}}$ and is inversely associated with body fat, demonstrating a link between insulin sensitivity, metabolic flexibility, aerobic capacity, and fatness (18,23). Sleep RQ on a standard 35% fat diet was inversely correlated with insulin sensitivity, pointing to a greater reliance on glucose as a fuel in insulin-insensitive subjects. This is consistent with previous reports (18) that measured fasting fat oxidation before a clamp in limb-balance studies. In our population, fasting fat oxidation during a clamp was not related to insulin sensitivity or body fat but was related to fasting FFAs. Taken together, these data demonstrate that defects in metabolic flexibility are present in healthy young men and mirror the defects seen in older subjects with obesity and type 2 diabetes (18).

There are several explanations for an impaired adaptive response to a HFD. First, it is possible that the fat oxidative machinery is not sufficient to match the fat load. Our results are consistent with this hypothesis. We found that mtDNA content in skeletal muscle was the best predictor of sleep fat oxidation after 3 days of a HFD. Second, an iso-energetic, high-fat, low-carbohydrate diet is known to increase fat oxidation (41) and decrease glucose metabolism in skeletal muscle (42). It is possible that differences in the ability of muscle to switch the substrate oxidative machinery in response to a HFD may contribute to the variability in adaptation to a HFD. Third, the endocrine signal(s) necessary to activate fat oxidation might be delayed or absent. Our results do not support this view, since adaptation to a HFD was not associated with changes in adiponectin, leptin, or FFAs, factors that are known to influence fat oxidation in skeletal muscle (43).

Mitochondrial mass, structure, and function are altered in insulin resistance (44,45). Defects of mitochondria are believed to contribute to impaired fat oxidation and to the accumulation of intramyocellular lipid intermediates, which contribute to the pathogenesis of insulin resistance (16). Mitochondrial dysfunction in the elderly and in the offspring of diabetic patients is well documented (24,46,47). Ritov et al. (45) demonstrated a reduction in mtDNA content in skeletal muscle in obese and type 2 diabetic subjects. Similarly, we show that mtDNA content in skeletal muscle is inversely correlated with BMI and body fat and is positively associated with insulin sensitivity, metabolic flexibility, aerobic capacity, and maximal HFD-induced fat oxidation, measured as sleep RQ, in healthy young adults. Skeletal muscle mitochondrial content may link these metabolic phenotypes, supporting the hypothesis that reduced mitochondrial mass is a common underlying disorder of inflexible/inadaptable/insulin-resistant skeletal muscle, before the known changes associated with aging and the development of type 2 diabetes. The importance of a genetic predisposition in this scenario is supported by our observation that a decrease in mtDNA content is observed in subjects with a family history of diabetes, consistent with findings in Mexican Americans with a family history of diabetes (A. Civitarese, unpublished data). Intrinsic defects of mitochondrial mass and function might be further diminished by environmental factors, such as a sedentary lifestyle, and a long-term HFD via mechanisms involving lipid peroxidation (48), decreased mitochondrial transcription factors (6), and reduced oxidative capacity (5,6). These cellular changes lead to type 2 diabetes when the β-cell ultimately fails.

There is good evidence to support a role for a genetic predisposition in the reduced fat-oxidative capacity of skeletal muscle. Muscle cells from morbidly obese subjects and type 2 diabetic subjects display defects in fat oxidation in vitro (49,50). We previously found that differences in fat oxidation are present in primary myotubes from young healthy individuals and correlate with clinical phenotypes of their donors, such as insulin sensitivity and metabolic flexibility (23). The estimated heritability of 24-h RQ is 20–30% (51). Individuals with a family history of diabetes were shown to have lower fat oxidation and higher RQ (52). Here, we demonstrate for the first time that maximal HFD-induced fat oxidation was significantly reduced in subjects with a family history of diabetes. This supports the hypothesis that patterns of fat oxidation are genetically or epigenetically determined, which is consistent with our in vitro studies (23). The lower rate of maximal HFD-induced fat oxidation was paralleled by a reduction in metabolic flexibility. The differences in metabolic phenotypes between subjects with and without a family history of diabetes were independent of body fat, suggesting that factors other than fatness per se are important in the pathogenesis of insulin resistance.

Regular exercise increases mitochondrial biogenesis and aerobic fitness. As such, the observed differences in mtDNA between subjects with a positive versus negative family history of diabetes could be due to differences in habitual physical activity. Using triaxial accelerometry, we did not observe differences in habitual physical activity level between subjects with a positive or negative family history of diabetes. $V_{O_{2max}}$ and the capacity to increase $V_{O_{2max}}$ with increasing physical activity both have a strong genetic component (53,54). One of the most striking differences between men with a positive versus negative family history of diabetes was a decrease in mtDNA and maximal fat oxidative capacity. The difference in maximal fat oxidative capacity remained significant after adjusting for $V_{O_{2max}}$. This suggests that other factors with a genetic component contribute to mitochondrial mass and, therefore, the reduced capacity to adapt to a HFD in young men with a family history of diabetes.

In conclusion, our results link metabolic inflexibility of skeletal muscle, impaired adaptation to a HFD, and reduced mtDNA content in skeletal muscle. The constellation of these metabolic derangements with reduced mtDNA content in skeletal muscle in subjects with a family history of diabetes might act in concert with a HFD and low physical activity, favoring lipid/energy storage and progression to obesity and diabetes.

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